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Construction and characterization of Escherichia coli O157:H7 strains expressing firefly luciferase and green fluorescent protein and their use in survival studies.
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ABSTRACT: The firefly (Photinus pyralis) luciferase (luc) gene on plasmid vector pBESTluc and the Aequorea victoria green fluorescent protein (gfp) gene on plasmid vector pGFP were introduced into strains of Escherichia coli O157:H7. The recombinant E. coli strains were indistinguishable from their parent strains in biochemical and immunological assays and in a multiplex PCR reaction. There was no significant difference in the growth kinetics of the luc-bearing recombinants and the parent strains. At 37degreeC all of the recombinant strains maintained the vectors and expressed luciferase and the green fluorescent protein when grown both with and without antibiotic selection. Individual colonies of luc-bearing E. coli strains were readily luminescent in the dark after being sprayed

with a solution of 1 mM beetle luciferin. The recombinants containing pGFP emitted bright green fluorescence when excited with UV light and the addition of any other proteins, substrates, or cofactors was not required. The green fluorescent protein-expressing E. coli O157:H7 strains were used in studies examining the survival of the organism in apple cider and in orange juice. In apple cider the organism declined to undetectable levels in 24 days at refrigeration temperature while in orange juice the strains survived with only small decreases in number during the 24-day sampling period. These recombinant E. coli O157:H7 strains, containing readily identifiable and stable markers, could be useful as positive controls in microbial assays as well as in studies monitoring bacterial survival and the behavior of E. coli O157:H7 in foods and in a food processing environment.

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Keywords

luciferase recombinant Escherichia food marker
fluorescent protein recombinant Escherichia food marker

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Apple juice
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Genetic engineering
Orange juice
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Proteins (specific proteins and subclasses)
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